FEBS Letters 581 (2007) 5255–5258

Aromatic structure of Tyrosine-92 in the extrinsic PsbU protein of red algal Photosystem II is important for its functioning

Akinori Okumura[a], Masanori Sano[b], Takehiro Suzuki[c,d], Hiroyasu Tanaka[b], Ryo Nagao[b], Katsuyoshi Nakazato[a], Masako Iwai[e], Hideyuki Adachi[f], Jian-Ren Shen[f], Isao Enami[b,*]

[a] *Department of Integrated Sciences in Physics and Biology, College of Humanities and Sciences, Nihon University, Tokyo 156-8850, Japan*
[b] *Department of Biology, Faculty of Science, Tokyo University of Science, Kagurazaka 1-3, Shinjuku-ku, Tokyo 162-8601, Japan*
[c] *Biomolecular Characterization Team, Discovery Research Institute, RIKEN, Saitama 351-0198, Japan*
[d] *JST, CREST, Japan*
[e] *Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science, Chiba 278-8510, Japan*
[f] *Division of Bioscience, Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan*

Received 5 September 2007; revised 29 September 2007; accepted 9 October 2007

Available online 16 October 2007

Edited by Richard Cogdell

Abstract **PsbU is one of the extrinsic proteins in red algal Photosystem II (PSII) and functions to optimize the availability of Ca^{2+} and Cl^- cofactors for water oxidation. To determine the functional residue of PsbU, we constructed various PsbU mutants from a red alga *Cyanidium caldarium* and reconstituted these mutants with the red algal PSII. The results revealed that Tyr-92 of PsbU, especially its aromatic ring, was essential for maintaining its function. From the crystal structure of PSII, Tyr-92 is located close to Pro-340 of D1, suggesting that the aromatic ring of Tyr-92 interacts with the CH group of Pro-340 of D1, and this CH/π interaction is important for the optimal function of the Mn_4Ca-cluster.**

Keywords: PsbU; Extrinsic protein; Oxygen evolution; Photosystem II; Red algae

1. Introduction

Photosystem II (PSII) exists in thylakoid membranes from cyanobacteria to higher plants and catalyzes light-induced electron transfer leading to the water-splitting and oxygen evolution. PSII is consisted of around 20 subunits, among which there are 3–4 extrinsic proteins that function to maintain the stability and activity of the oxygen-evolving complex. PSIIs of cyanobacteria and most eukaryotic algae such as red algae, diatoms and brown algae contain three extrinsic proteins of 33 kDa (PsbO), cytochrome *c*550 (PsbV) and 12 kDa (PsbU), although green algal and *Euglena* PSIIs contain PsbP and PsbQ instead of PsbV and PsbU [1–5]. In addition, PSII purified from a red alga *Cyanidium caldarium* contained an additional extrinsic protein of 20 kDa encoded by the *psbQ′* gene which has a low homologue with the *psbQ* genes of green algae and higher plants [6,7]. Among these extrinsic proteins, PsbU was found to function in optimizing the availability of Ca^{2+} and Cl^- cofactors for water oxidation in PSII from the red alga *C. caldarium* [6,8]. The red algal PsbU is consisted of 93 amino acids with a molecular mass of 10513 Da [9]. To identify the functional domains of PsbU, we have prepared the PsbU proteins lacking several amino acid residues from the N-terminus and/or C-terminus by limited proteolysis and directed mutagenesis, and examined their binding and functional properties by reconstitution experiments. Our results indicated that two residues (Ile-91 and/or Tyr-92) from the lysine residue of the C-terminus of PsbU constituted an important domain for minimizing the Ca^{2+} and Cl^- requirement of oxygen evolution [8].

In order to identify which residue in the C-terminal domain is required for the function of PsbU, we deleted one or two residues from the C-terminus and reconstituted the mutant protein with red algal PSII retaining PsbO, PsbQ′ and PsbV. Analysis of oxygen-evolving activities in the absence or presence of NaCl and $CaCl_2$ of the reconstituted PSII revealed that Tyr-92 was important for its functioning. To examine the role of Tyr-92 in more detail, we constructed a series of mutants by replacing Tyr-92 with various amino acids. These results indicated that the aromatic structure of Tyr-92 in PsbU was essential to maintain its functional structure. Based on the present results and the recent crystal structure of cyanobacterial PSII [10], the functional structure of PsbU in relation to its interaction with intrinsic proteins of PSII was discussed.

2. Materials and methods

2.1. Preparation of oxygen-evolving PSII from the red alga

Oxygen-evolving PSII complexes were prepared from an acidophilic and thermophilic red alga, *C. caldarium*, according to Enami et al. [2] with slight modifications. Thylakoid membranes isolated from *C. caldarium* cells were solubilized with 1.3% *n*-dodecyl-β-D-maltoside and then loaded onto a DEAE-Sepharose CL-6B column to purify the red algal PSII.

2.2. Expression and purification of various mutants of PsbU

Mutant proteins of PsbU either lacking one or two residues of the C-terminus, or with its Tyr-92 replaced by Thr, Phe, Asp, Arg, Ile, Gly, Trp, Pro or His were generated by the PCR method, expressed in the host cell BL21(DE3) and purified as described previously [8].

2.3. Reconstitution experiments

The red algal PSII was treated with 1 M $CaCl_2$ to remove all of the four extrinsic proteins. The $CaCl_2$-treated PSII was incubated with various PsbU mutants, together with PsbO, PsbQ′ and PsbV, for 30 min at 0 °C in the dark, as described previously [8]. The reconstituted PSII

*Corresponding author. Fax: +81 3 3260 0322.
E-mail address: enami@rs.noda.tus.ac.jp (I. Enami).

0014-5793/$32.00
doi:10.1016/j.febslet.2007.10.015

was centrifuged at 40 000 × *g* for 20 min after addition of 10% polyethylene glycol 6000. The precipitate was suspended in a medium of 25% glycerol and 50 mM MES–NaOH (pH 6.5) (buffer A), and the binding and functional properties of native and mutant PsbU were analyzed by SDS–PAGE and oxygen evolution measurement.

2.4. SDS–PAGE

Samples were solubilized with 5% lithium lauryl sulfate and 75 mM dithiothreitol. The solubilized samples were applied to an SDS–polyacrylamide gel containing a gradient of 16–22% polyacrylamide and 7.5 M urea.

2.5. Assay of oxygen evolution

Oxygen evolution was measured with a Clark-type oxygen electrode at 25 °C in buffer A with phenyl-*p*-benzoquinone as electron acceptor, in the absence or presence of 10 mM NaCl or 5 mM $CaCl_2$.

3. Results

3.1. Tyr-92 is important for the functioning of PsbU in optimizing the availability of Ca^{2+} and Cl^- cofactors for water oxidation

As described previously [6,8], the four extrinsic proteins in red algal PSII were completely removed by treatment with 1 M $CaCl_2$ (lane 2 in Fig. 1). When the $CaCl_2$-treated PSII was reconstituted with PsbO, PsbQ′ and PsbV, these three extrinsic proteins rebound to the $CaCl_2$-treated PSII (lane 3 in Fig. 1). Further reconstitution with native (lane 4) or mutant PsbU either lacking one (lane 5) or two residues (lane 6) of the C-terminus led to the complete binding of the native PsbU protein as well as its two mutants (Fig. 1), indicating that the binding abilities of PsbU are conserved in the two mutant proteins. This is consistent with our previous observation that even the removal of both of nine residues from the N-terminus and 10 residues from the C-terminus simultaneously by chymotrypsin treatment did not affect its stoichiometric binding to PSII [8].

In spite of complete rebinding of the two PsbU mutants, the recovery of oxygen evolution was largely different between the PsbU mutants lacking one or two residues from the C-terminus. As shown in Table 1, the native PSII of red alga showed oxygen-evolving activities of 3095, 3144 or 3190 μmol O_2 per mg Chl per h in the absence or presence of 10 mM NaCl or 5 mM $CaCl_2$, respectively. When the four extrinsic proteins were removed by treatment with 1 M $CaCl_2$, the activity was almost completely lost. By reconstitution of the $CaCl_2$-treated PSII with PsbO, PsbQ′ and PsbV, the oxygen-evolving activity recovered to 3%, 36% or 49% of that of untreated PSII, respectively, in the absence or presence of 10 mM NaCl or 5 mM $CaCl_2$. When the native PsbU protein was reconstituted together with the other three extrinsic proteins, the activity was recovered to 66%, 67% or 69%, respectively. This indicates that PsbU functions to optimize the availability of Ca^{2+} and Cl^- cofactors for water oxidation in the red algal PSII, in agreement with our previous results [6,8]. The recovery of oxygen-evolving activity by reconstitution with the PsbU mutant lacking one residue of the C-terminus (R1-Y92) was similar to that of reconstitution with native PsbU (R1-K93). In contrast, no recovery of oxygen-evolving activity was observed by reconstitution with the PsbU mutant lacking two residues of the C-terminus (R1-I91). These results clearly indicate that the second residue from the C-terminus of PsbU, Tyr-92, is important for its functioning in optimizing the availability of Ca^{2+} and Cl^- cofactors for water oxidation.

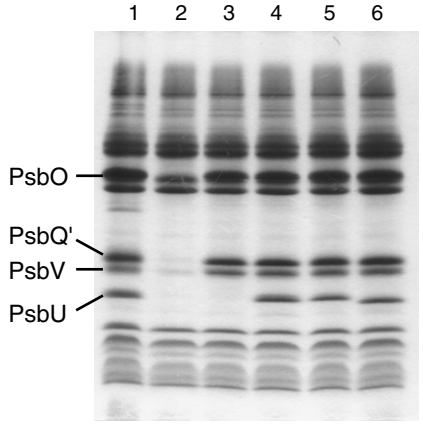

Fig. 1. Reconstitution of red algal PSII with various extrinsic proteins. Lane 1, control PSII; lane 2, $CaCl_2$-treated PSII; lanes 3–6, $CaCl_2$-treated PSII reconstituted with PsbO, PsbQ′ and PsbV (lane 3), PsbO, PsbQ′ and PsbV plus native PsbU (R1-K93) (lane 4), PsbO, PsbQ′ and PsbV plus PsbU mutant lacking one residue from the C-terminus (R1-Y92) (lane 5), and PsbO, PsbQ′ and PsbV plus PsbU mutant lacking two residues from the C-terminus (R1-I91) (lane 6).

3.2. Aromatic structure of Tyr-92 in PsbU is essential for maintaining its functional structure

To determine which structure of Tyr-92 in PsbU is essential for maintaining its functioning, we constructed a series of mutants by replacing Tyr-92 with Thr (Y92T), Phe (Y92F), Asp (Y92D), Arg (Y92R), Ile (Y92I), Gly (Y92G), Trp (Y92W), Pro (Y92P) or His (Y92H). All of these mutants were able to rebind to the $CaCl_2$-treated PSII retaining the other three extrinsic proteins completely (data not shown). This indicates that there are no effects on the binding ability of PsbU by replacing Tyr-92 with various amino acids, in agreement with the above results that even deletion of Tyr-92 did not affect its binding ability to PSII.

Table 2 shows the recovery of oxygen-evolving activity by rebinding of these PsbU mutants. No recovery was observed when the Y92T mutant was reconstituted together with the other three extrinsic proteins, indicating that the hydroxyl group of Tyr-92 in PsbU is not responsible for its functioning. In contrast, reconstitution with the Y92F mutant showed a high recovery of activity which was comparable with that of PSII reconstituted with the native PsbU protein. When Y92D, Y92R, Y92I or Y92G mutant was reconstituted, no recovery was observed. These results clearly indicate that the aromatic structure of Tyr-92 in PsbU is essential for maintaining its functional structure.

In order to determine whether heterocyclic amino acids or imino acid are able to function to maintain the functional structure of PsbU instead of aromatic amino acids, we examined the oxygen-evolving activity of PSII reconstituted with PsbU mutants with its Tyr-92 replaced by Trp (Y92W), Pro (Y92P) or His (Y92H). Although the reconstitution of Y92F recovered the oxygen-evolving activity as described above, no recovery was observed in $CaCl_2$-treated PSII reconstituted with Y92W or Y92P (Table 2). In contrast, a partial recovery was obtained in the $CaCl_2$-treated PSII reconstituted with Y92H, suggesting that the imidazole ring of His partially func-

Table 1
Oxygen evolution of the $CaCl_2$-treated PSII reconstituted with native PsbU (R1-K93) or PsbU mutants lacking one (R1-Y92) or two residues (R1-I91) from C-terminus, together with the other three extrinsic proteins

	Oxygen evolution (μmol O_2/mg Chl/h)					
	−Ion	(%)	+10 mM NaCl	(%)	+5 mM $CaCl_2$	(%)
Untreated PSII	3095 ± 27	(100)	3144 ± 38	(100)	3190 ± 19	(100)
$CaCl_2$-treated PSII	31 ± 5	(1)	37 ± 7	(1)	124 ± 26	(4)
+PsbO + PsbQ′ + PsbV	82 ± 9	(3)	1124 ± 45	(36)	1576 ± 49	(49)
+PsbO + PsbQ′ + PsbV + PsbU (R1-K93)	2051 ± 38	(66)	2091 ± 33	(67)	2186 ± 67	(69)
+PsbO + PsbQ′ + PsbV + PsbU (R1-Y92)	1934 ± 69	(62)	1971 ± 84	(63)	2040 ± 70	(64)
+PsbO + PsbQ′ + PsbV + PsbU (R1-I91)	87 ± 4	(3)	1103 ± 45	(35)	1567 ± 54	(49)

The values shown are means ± standard error from three separate measurements.

Table 2
Oxygen evolution of the $CaCl_2$-treated PSII reconstituted with native PsbU or PsbU mutants of Y92T, Y92F, Y92D, Y92R, Y92I, Y92G, or Y92F, Y92W, Y92P, Y92H, together with the other three extrinsic proteins

	Oxygen evolution (μmol O_2/mg Chl/h)					
	−Ion	(%)	+10 mM NaCl	(%)	+5 mM $CaCl_2$	(%)
Untreated PSII	3140 ± 28	(100)	3206 ± 12	(100)	3259 ± 39	(100)
$CaCl_2$-treated PSII	35 ± 8	(1)	44 ± 9	(1)	190 ± 12	(6)
+PsbO + PsbQ′ + PsbV	85 ± 20	(3)	1188 ± 81	(37)	1493 ± 78	(46)
+PsbO + PsbQ′ + PsbV + native PsbU	2026 ± 42	(65)	2107 ± 49	(66)	2200 ± 86	(68)
+PsbO + PsbQ′ + PsbV + PsbU (Y92T)	83 ± 20	(3)	1178 ± 63	(37)	1448 ± 80	(44)
+PsbO + PsbQ′ + PsbV + PsbU (Y92F)	1884 ± 29	(60)	1959 ± 57	(61)	2032 ± 44	(62)
+PsbO + PsbQ′ + PsbV + PsbU (Y92D)	82 ± 19	(3)	1209 ± 34	(38)	1537 ± 52	(47)
+PsbO + PsbQ′ + PsbV + PsbU (Y92R)	76 ± 21	(2)	1198 ± 33	(37)	1470 ± 59	(45)
+PsbO + PsbQ′ + PsbV + PsbU (Y92I)	80 ± 21	(3)	1246 ± 33	(39)	1504 ± 85	(46)
+PsbO + PsbQ′ + PsbV + PsbU (Y92G)	90 ± 19	(3)	1199 ± 72	(37)	1515 ± 67	(46)
+PsbO + PsbQ′ + PsbV + PsbU (Y92F)	1852 ± 47	(59)	1988 ± 39	(62)	2183 ± 49	(67)
+PsbO + PsbQ′ + PsbV + PsbU (Y92W)	126 ± 8	(4)	1154 ± 51	(36)	1695 ± 38	(52)
+PsbO + PsbQ′ + PsbV + PsbU (Y92P)	124 ± 10	(4)	1162 ± 74	(36)	1792 ± 84	(55)
+PsbO + PsbQ′ + PsbV + PsbU (Y92H)	942 ± 18	(30)	1879 ± 52	(59)	2151 ± 39	(66)

The values shown are means ± standard errors from three separate measurements.

tions to maintain the functional structure of PsbU instead of the aromatic ring of Tyr-92.

4. Discussion

In this study, we found that the aromatic structure of Tyr-92 in red algal PsbU is important for its functioning in optimizing the availability of Ca^{2+} and Cl^- cofactors for water oxidation. As described previously [8], the second amino acid from the C-terminus, Tyr, is completely conserved among all species including cyanobacteria, red algae and diatoms, supporting that this Tyr residue in PsbU is essential for its functioning among various species.

Recent crystallographic investigations of cyanobacterial PSII have provided medium-resolution structures (3.0–3.7 Å) that explain the general arrangement of the protein matrix and cofactors [10–12]. We examined the location of Tyr-92 of PsbU in the published 3.0 Å resolution structure of cyanobacterial PSII [10] and searched for the residues of intrinsic proteins that may interact with Tyr-92 of PsbU. As shown in Fig. 2, Tyr-103 of cyanobacterial PsbU, corresponding to Tyr-92 of the red algal PsbU, was located at a distance of 3.4 Å to Pro-340 of D1, suggesting a possible interaction between them. It has to be mentioned that there remains a possibility that the structure of red algal PSII is different from

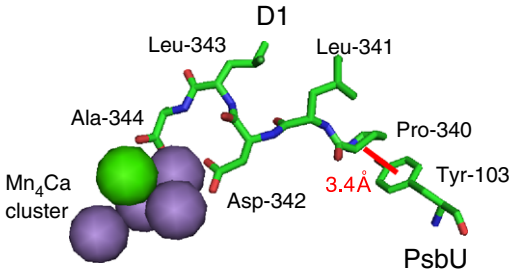

Fig. 2. Structure of the Mn_4Ca-cluster and the surrounding C-terminal residues of D1 together with Tyr-103 of cyanobacterial PsbU. The structure was drawn based on the published 3.0 Å resolution structure of cyanobacterial PSII (PDB ID: 2AXT). Tyr-103 of the cyanobacterial PsbU corresponding to Tyr-92 of red algal PsbU is located at a distance of 3.4 Å to Pro-340 of D1, suggesting their possible interaction. For clarity, only residues of D1 closely related to Pro-340 and the Mn_4Ca-cluster were shown.

that of cyanobacterial PSII, because an extra extrinsic protein, PsbQ′, is present in red algal PSII but not in cyanobacterial PSII. Recent single particle image analysis, however, revealed that the location of PsbU in the red algal PSII from *C. caldarium* was similar to that of cyanobacterial PSII [13]. Furthermore, we have shown previously that the red algal PsbU protein can functionally bind to the cyanobacterial PSII from

Thermosynechococcus vulcanus [14]. These facts indicate that the overall structure of red algal PSII, at least with respect to the interaction of PsbU with PSII intrinsic proteins, is very similar to that of cyanobacterial PSII, except for the presence of PsbQ′ in the former, but not in the latter, PSII.

It has been reported that interaction between CH groups of various compounds and the π-electron systems of aromatic amino acid residues (CH/π interaction) plays an important role for maintaining functional structure of proteins, such as ligand-recognition function of carbohydrate-binding proteins [15], enzymatic activity of RNase [16], or recognition of DNA and RNA molecules by proteins [17]. The attraction and directionality of the CH/π interaction were examined in the benzene–methane complex, and the interaction energy was estimated to be −1.45 kcal/mol and to have a minimum at 3.4 Å [18]. These results suggest also a CH/π interaction between PsbU and D1, namely, the π-electron systems of the aromatic ring of Tyr-92 in red algal PsbU (Tyr-103 in cyanobacterial PsbU) interacts with the CH group of Pro-340 in D1.

Pro-340 of D1 is located in the C-terminal region of D1 where a number of residues are involved directly or indirectly in ligating the Mn_4Ca-cluster, including the two important C-terminal residues Asp-342 and Ala-344. Site-directed mutagenesis studies of these residues using a cyanobacterium *Synechocystis* sp. PCC 6803 have shown that Asp-342 modulated the calcium requirement of the water oxidation, and that the α-COO^- group of Ala-344 was ligated to the Mn_4Ca-cluster [19,20]. Thus, it is likely that the proposed CH/π interaction between Tyr-92 of PsbU and Pro-340 of D1 affected the structure of the C-terminal region of D1 required for maintaining the optimum structure of the Mn_4Ca-cluster in the absence of the Ca^{2+} and Cl^- cofactors.

Acknowledgements: This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Technology of Japan (MEXT) to I.E. (18570049) and Scientific Research on Priority Areas from the MEXT to K.N. and J.-R.S. (16087102).

References

[1] Shen, J.-R. and Inoue, Y. (1993) Binding and functional properties of two new extrinsic components, cytochrome *c*-550 and a 12 kDa protein, in cyanobacterial photosystem II. Biochemistry 32, 1825–1832.
[2] Enami, I., Murayama, H., Ohta, H., Kamo, M., Nakazato, K. and Shen, J.-R. (1995) Isolation and characterization of a photosystem II complex from the red alga *Cyanidium caldarium*: association of cytochrome *c*-550 and a 12 kDa protein with the complex. Biochim. Biophys. Acta 1232, 208–216.
[3] Enami, I., Suzuki, T., Tada, O., Nakada, Y., Nakamura, K., Tohri, A., Ohta, H., Shen, J.-R. and Inoue, I. (2005) Distribution of the extrinsic proteins as a potential marker for the evolution of photosynthetic oxygen-evolving photosystem II. FEBS J. 272, 5020–5030.
[4] Suzuki, T., Minagawa, J., Tomo, T., Sonoike, K., Ohta, H. and Enami, I. (2003) Binding and functional properties of the extrinsic proteins in oxygen-evolving Photosystem II particle from a green alga, *Chlamydomonas reinhardtii* having his-tagged CP47. Plant Cell Physiol. 44, 76–84.
[5] Suzuki, T., Tada, O., Makimura, M., Tohri, A., Ohta, H., Yamamoto, Y. and Enami, I. (2004) Isolation and characterization of oxygen-evolving Photosystem II complexes retaining the PsbO, P and Q proteins from *Euglena gracilis*. Plant Cell Physiol. 45, 1168–1175.
[6] Enami, I., Kikuchi, S., Fukuda, T., Ohta, H. and Shen, J.-R. (1998) Binding and functional properties of four extrinsic proteins of photosystem II from a red alga, *Cyanidium caldarium*, as studied by release-reconstitution experiments. Biochemistry 37, 2787–2793.
[7] Ohta, H., Suzuki, T., Ueno, M., Okumura, A., Yoshihara, S., Shen, J.-R. and Enami, I. (2003) Extrinsic proteins of photosystem II: an intermediate member of PsbQ protein family in red algal PS II. Eur. J. Biochem. 270, 4156–4163.
[8] Okumura, A., Ohta, H., Inoue, Y. and Enami, I. (2001) Identification of functional domains of the extrinsic 12 kDa protein in red algal PSII by limited proteolysis and directed mutagenesis. Plant Cell Physiol. 42, 1331–1337.
[9] Ohta, H., Okumura, A., Okuyama, S., Akiyama, A., Iwai, M., Yoshihara, S., Shen, J.-R., Kamo, M. and Enami, I. (1999) Cloning, expression of the *psb*U gene, and functional studies of the recombinant 12-kDa protein of photosystem II from a red alga *Cyanidium caldarium*. Biochem. Biophys. Res. Commun. 260, 245–250.
[10] Loll, B., Kern, J., Saenger, W., Zouni, A. and Biesiadka, J. (2005) Towards complete cofactor arrangement in the 3.0 Å resolution structure of photosystem II. Nature 438, 1040–1044.
[11] Kamiya, N. and Shen, J.-R. (2003) Crystal structure of oxygen-evolving photosystem II from *Thermosynechococcus vulcanus* at 3.7-Å resolution. Proc. Natl. Acad. Sci. USA 100, 98–103.
[12] Ferreira, K.N., Iverson, T.M., Maghlaoui, K., Barber, J. and Iwata, S. (2004) Architecture of the photosynthetic oxygen-evolving center. Science 303, 1831–1838.
[13] Gardian, Z., Bumba, L., Schrofel, A., Herbstova, M., Nebesarova, J. and Vacha, F. (2007) Organisation of Photosystem I and Photosystem II in red alga *Cyanidium caldarium*: Encounter of cyanobacterial and higher plant concepts. Biochim. Biophys. Acta 1767, 725–731.
[14] Enami, I., Iwai, M., Akiyama, A., Suzuki, T., Okumura, A., Katoh, T., Tada, O., Ohta, H. and Shen, J.-R. (2003) Comparison of binding and functional properties of two extrinsic components, cyt *c*550 and a 12 kDa protein, in cyanobacterial PSII with those in red algal PSII. Plant Cell Physiol. 44, 820–827.
[15] Muraki, M. (2002) The importance of CH/π interactions to the function of carbohydrate binding proteins. Protein Pept. Lett. 9, 195–209.
[16] Ohgi, K., Kudo, S., Takeuchi, M., Iwama, M. and Irie, M. (2000) Enzymatic properties of phenylalanine101 mutant enzyme of ribonuclease Rh from *Rhizopus niveus*. Biosci. Biotechnol. Biochem. 64, 2068–2074.
[17] Chakrabarti, P. and Samanta, U. (1995) CH/π interaction in the packing of the adenine ring in protein structures. J. Mol. Biol. 251, 9–14.
[18] Tsuzuki, S., Honda, K., Uchimaru, T., Mikami, M. and Tanabe, K. (2000) Origin of the attraction and directionality of the NH/π interaction: comparison with OH/π and CH/π interactions. J. Am. Chem. Soc. 122, 11450–11458.
[19] Chu, H.-A., Nguyen, A.P. and Debus, R.J. (1995) Amino acid residues that influence the binding of manganese or calcium to Photosystem II. 2. The carboxy-terminal domain of the D1 polypeptide. Biochemistry 34, 5859–5882.
[20] Mizusawa, N., Kimura, Y., Ishii, A., Yamanari, T., Nakazawa, S., Teramoto, H. and Ono, T. (2004) Impact of replacement of D1 C-terminal alanine with glycine on structure and function of photosynthetic oxygen-evolving complex. J. Biol. Chem. 279, 29622–29627.